

ANNUAL REPORT

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**“Linear and Circular Polarisation Vision in Stomatopod Crustaceans:
Information Processing and Signals to the Brain,
Secret Signals Between Animals and Contrast Enhancement.”**

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14. ABSTRACT This report covers polarization vision, information processing and communication in stomatopod crustaceans. Specifically, determine if there are polarization connection pathways in the neural processing systems, and conduct electrophysiology experiments to determine the anatomy of interconnection beneath the retina.				
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Overview

This funding has enabled us to achieve 4 main things:

- a) To make new discoveries about the strangest visual system known – the stomatopod eye. This is summarised below.
- b) Tsyr-Huei (Short) Chiou has begun a very successful Post Doctoral study period at The university of Queensland, transitioning from the lab. of close collaborator Tom Cronin.
- c) Prepare new equipment and methods for on-going work funded by AOARD/AFOSR.
- d) Allowed us to move into new areas and techniques aimed at addressing the core-questions of this work.

During the period of the grant, we also secured other joint funding to run an international workshop on New Perspectives in Polarised Light in Australia and results from this have been accepted as a special edition of The Philosophical Transactions of the Royal Society of London. Some of the results from this grant will be contained in that monograph, due out in 2010.

The initial rational from the work plan was as follows:

Rationale

We aim to further combine the results from two AFOSR/AOARD funded labs, one Australian and one American, to interpret polarisation vision, signals and the polarised light habitats of stomatopod crustaceans (mantis shrimps). Discoveries over the last three years have told us that, uniquely among known animals, stomatopods are capable of circular polarisation vision. The astonishing optical and physiological photoreceptor mechanisms for this are becoming clear. We have also learned much about stomatopod light habitats and the ways in which they produce polarised signals (both circular and linear Fig.1) to communicate in what appears to be a ‘secretly’ coded language. This next phase of research aims to discover how photoreceptors are combined or ‘wired up’ and how messages are processed and sent to the brain. We also aim to move towards potential applications of linear and circular polarisation vision in marine and other environments.

Much of this has been achieved with new results potentially touching the lives of most of mankind (at least those with or reliant on computers) through DVD and CD design improvement (see Nature Photonics article and publicity follow up, also Appendix 1 front cover here). We have paved the way to achieve more in the direction of neural processing, and as this report shows, have adopted new techniques to achieve this, over and above those suggested from the outset.

We still have not achieved great inroads into the processing of the information from the stomatopod retina, but this is well underway and the continuing Post Doctoral support from AOARD and AFOSR will ensure this continues.

We have at all times found the support of AOARD (and AFOSR) in all respects efficient, helpful and interested. Many thanks for it!

Objectives

(1) Study the neuronal pathways which involved in the processing of polarization visual information.

Similar to colour vision, polarized light based visual signals must be picked up by multiple photoreceptors. Through comparison, summation, and subtraction of the conditions from various photoreceptors, the neural system of stomatopod can thus work out the status of the polarized light reached their eyes. By combining electrophysiology with dye injection we were able to trace, successfully, the neural projection of photoreceptors of stomatopods (Kleinlogel and Marshall 2005, Kleinlogel et al. 2003, for review see: Marshall et al. 2007). However, since the chances of having dyes injected into neighbour photoreceptors are slim, such methods are inadequate for simultaneously tracking neuronal connections from multiple cells. To overcome such difficulties, we will adapt several neuronal staining methods that are commonly used for investigation of neuronal connections. By staining multiple photoreceptors at the same time, their targeted interneuron could be located for electrophysiological studies.

(2) Use electrophysiology methods to determine how polarized light information is processed in the visual pathway.

In crustacean, preliminary processing of visual signal occurred between photoreceptors and visual neuron piles cells that generate impulses. With potentially various types of neuron activities the signal processing in the visual pathway of stomatopod can only be studied by intracellular recordings. Here, we will conduct sharp electrode intracellular recordings from descending neurons (i.e. lamina, medulla, and lobula) of polarized light sensitive photoreceptors.

Status of Effort

In the past year, we have tested several methods for morphological investigation of neuron connections within the visual pathway of stomatopod. The method tested including using gene gun, dye-induced photo permeabilization and photo degeneration (Picaud, Peichl et al. 1990, Picaud, Wunderer et al. 1990, Picaud et al. 1988), scrape-loading (Elfouly et al. 1987), and osmotic lysis method (Okada and Rechsteiner 1982). What all the methods attempt is staining a small proportion of the nerves within the visual pathway in order to find potential connections. To gain a better understanding of the anatomy of the nervous system of stomatopod, we have also started to use high resolution (down to 10 μm) MRI (Magnetic Resonance Imaging) to scans.

On the practical side, in the electrophysiology lab, we have both re-furbished the hardware and also re-write the controlling program. By introducing better stepper motor and stepper motor controller, we gain better control on the light stimulus we are using. Another critical upgrade of the system is that we migrate from MS-DOS based program to a Windows based program. Take advantage of multitasking/multithreading ability of windows based system, our new program is able to record both the slow potential generated by cells such as photoreceptors and those producing fast impulses. In addition, the program was developed on Microsoft .net framework, which allows easy modification and extension of new function. The program and operation manual is available on request.

We have conducted two field trips for collecting stomatopods. In both trips, we collect animals and bring them back to lab for electrophysiology and behavioural experiments. In addition, we have collaborated with Profs Thomas Cronin and Roy Caldwell at Lizard Island Research Station. In this trip we were able to fix eye samples of different species as well as various development stages and brought them back for further morphological examination and comparison.

Accomplishments/New Findings

Among the methods used for tracing visual pathways, we have successfully loaded fluorescent dyes into a small group of photoreceptors within the mid-band rows 5 and 6 and able to trace their relations (Figure 1). Within these images, we have found unexpected connections between photoreceptors of different rows in the mid-band. To further confirm such connection, we will be conducting electron microscopy examination in this region.

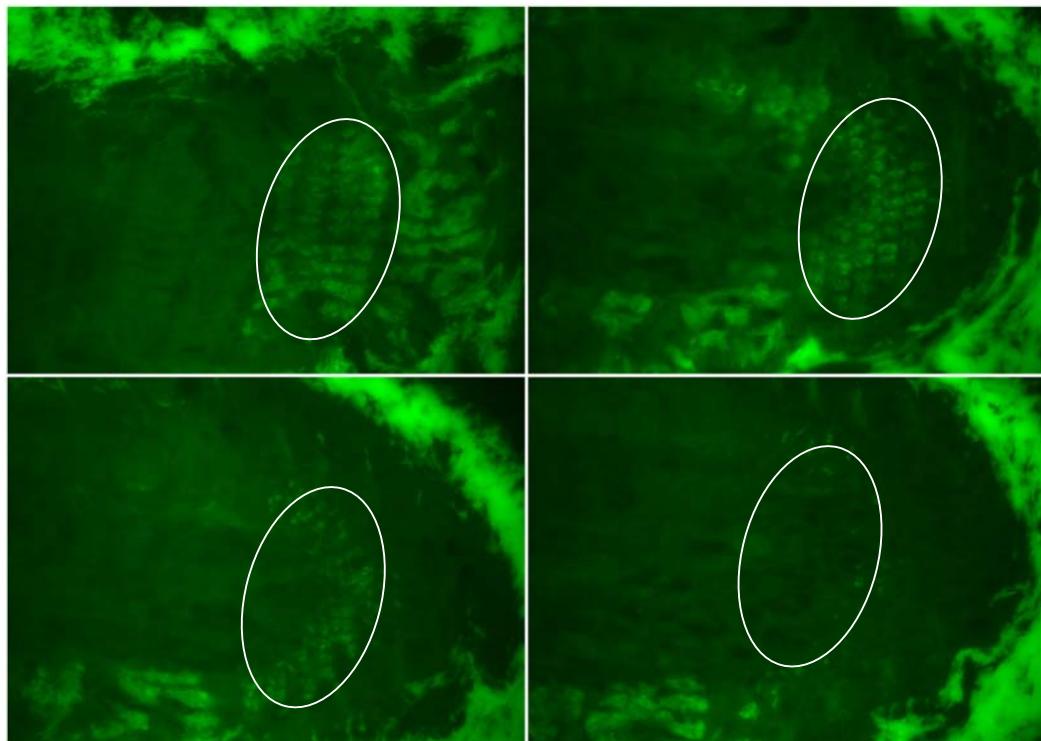


Figure 1. Serial section of the eyestalk of *Gonodactylus smithii* showing the projection of mid-band rows 5 and 6 photoreceptors in the lamina region. Note that the size, shape and number of axon endings changes dramatically between sections (green fluorescent stained areas within oval). This result indicates only part of the photoreceptors projected to the proximal end of the lamina.

In a new venture, we have conduct MRI scanning on the eyes of three different species of stomatopods, *Haplosquilla trispinosa*, *Gonodactylus smithii*, and *G. chiragra*. By experimenting with different contrast agents, we were able to visualize

the organization of visual neuropils in the eyestalk (Figure 2). Based on the stacks of images, three dimension reconstructions of the visual neuropils can be easily visualized and this reconstruction is underway.

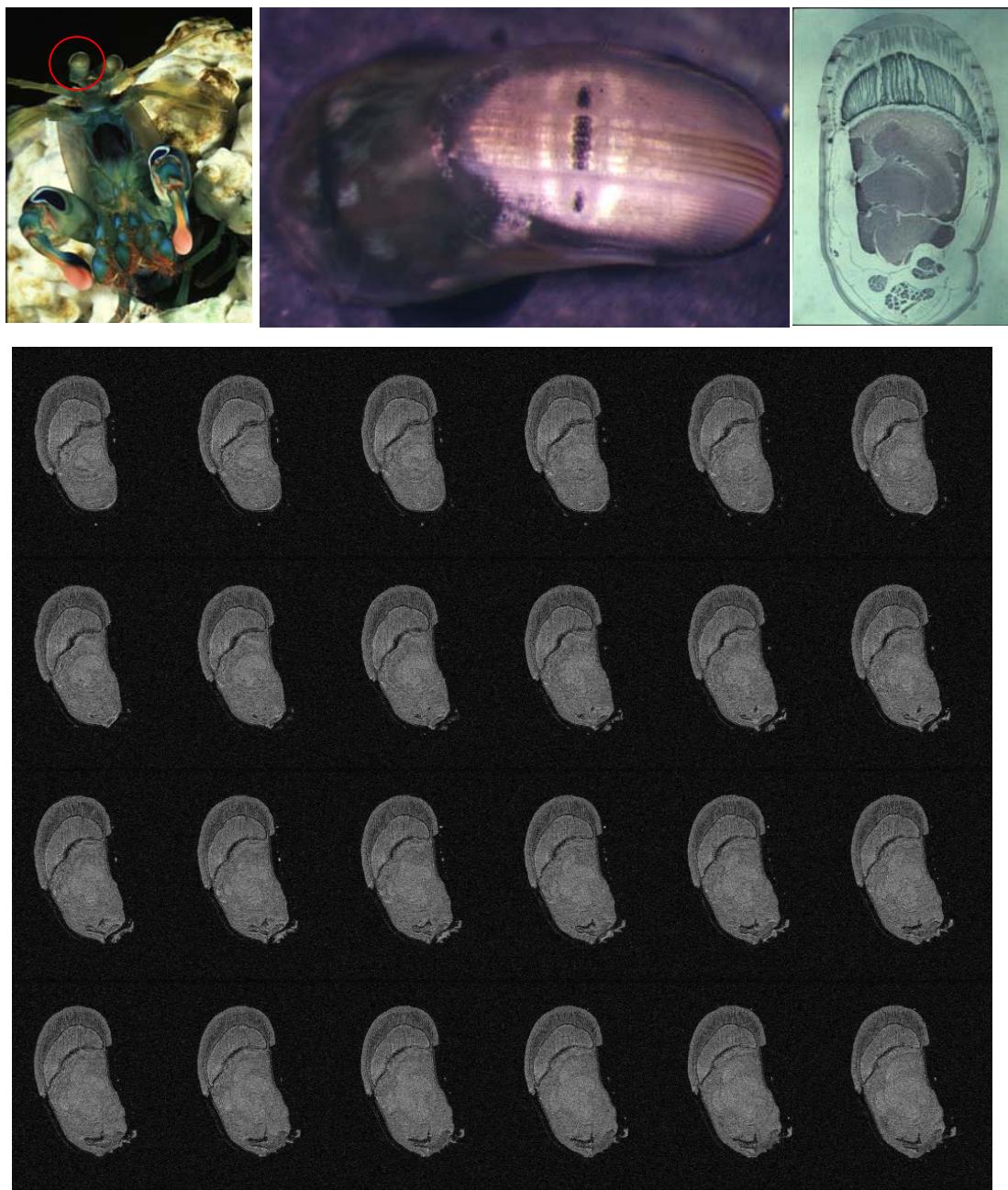


Figure 2. Top row - Gonodactyloid stomatopod displaying from hole on the reef. Excised eye circled red in whole animal. Standard histological section showing retina and neuropils. Bottom - Series of MRI images obtained from the eye stalk of *G. chiragra*. Visual neuropils and photoreceptors are a lighter grey colour in MRI scan.

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2. Chiou, T.-H., S. Kleinlogel, T. W. Cronin, R. L. Caldwell, B. Loeffler, A. Siddiqi, A. Goldizen, and J. Marshall. (2008). Circular polarization vision in a stomatopod crustacean. *Curr. Biol.* 18, 429-434.
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6. Kleinlogel S. and Marshall N.J. (In Press November 2009 - Front Cover) Ultraviolet polarisation sensitivity in the stomatopod crustacean *Odontodactylus scyllarus*. *J. Comp. Physiol. A* (NB this publication is not from this funding block but from previous AOARD supported grants for this work).

Submitted Manuscripts

Cronin, T. W., T.-H. Chiou, R. L. Caldwell, N. Roberts, and J. Marshall. (2009). Polarization signals in mantis shrimps. In *Polarization Sciences and Remote Sensing IV*, J. A. Shaw and J. S. Tyo, eds., Proc. SPIE. 7461, 74610C.

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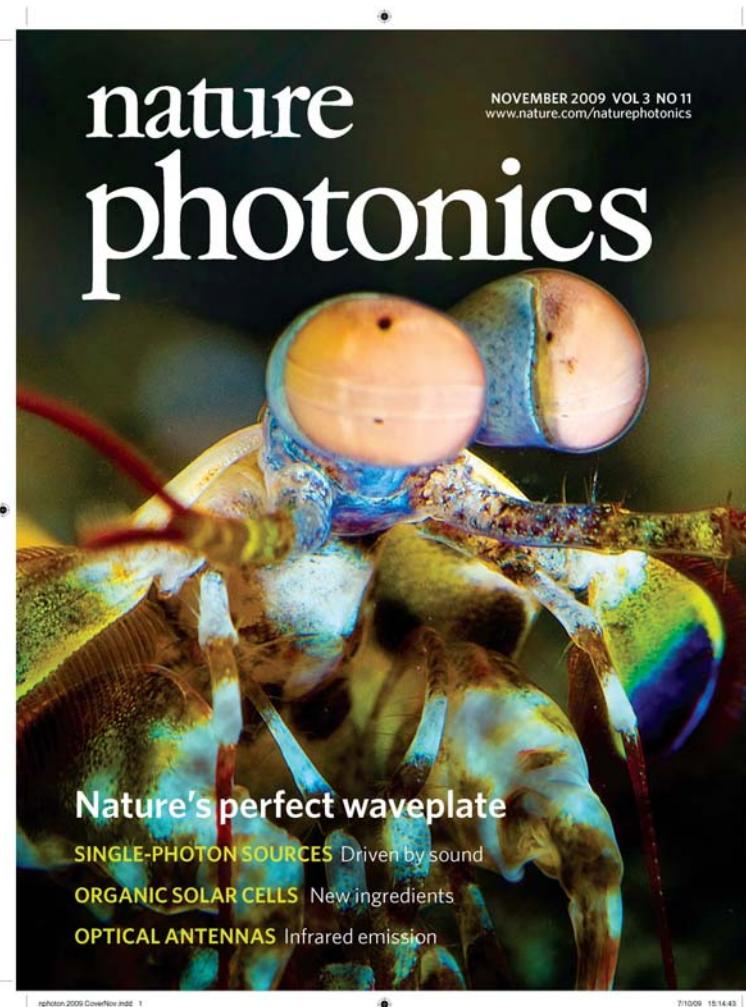
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Elfouly MH, Trosko JE and Chang CC. 1987. Scrape-Loading and Dye Transfer - a Rapid and Simple Technique to Study Gap Junctional Intercellular Communication. *Exp Cell Res.* 168(2):422-430.

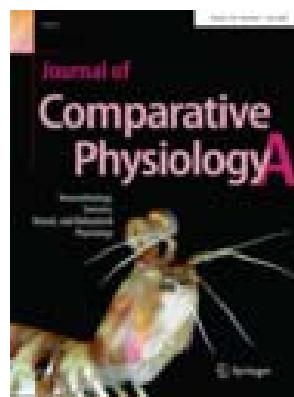
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Appendix 1

Current Front cover for Nature Photonics. <http://www.nature.com/nphoton/index.html>



Current Front cover for Journal of Comparative Physiology A also. (Don't have high resolution image yet). <http://www.springer.com/life+sci/zoology/journal/359>



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Nature News 461:1177

ARS Technica website <http://arstechnica.com/science/news/2009/11/a-crusty-eye-sees-curly-light.ars>

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